Volume 215, number 1, 9–12 FEB 04627 May 1987

ATP-sensitive K^+ channels in human isolated pancreatic B-cells

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Received 23 February 1987

Glucose-stimulated insulin release from rodent pancreatic B-cells is thought to be initiated by the closing of ATP-sensitive K^+ channels in the plasma membrane as a consequence of glucose metabolism. We have identified an ATP-sensitive K^+ channel in membrane patches excised from human B-cells which is similar to that found in rodent B-cells in conductance, kinetics, ATP sensitivity and its inhibition by sulphonylureas. In man, the ATP-sensitive K^+ channel may also have a central role in glucose-stimulated insulin secretion and may be (linked to) the receptor for the hypoglycemic sulphonylureas.

ATP sensitivity; K^+ channel; Patch clamp; Insulin secretion; (Pancreatic B-cell)

1. INTRODUCTION

The recent application of patch-clamp techniques to isolated pancreatic B-cells has contributed to our understanding of the mechanism by which glucose regulates insulin secretion. These studies have shown that the ATP-sensitive K^+ channel is the major determinant of the B-cell resting membrane potential in glucose-free solution [1,2]. In response to an increase in the external glucose concentration the channel closes, producing a depolarisation of the B-cell which leads to increased entry of Ca^{2+} into the cell and thereby insulin secretion [1,2]. The ATP-sensitive K^+ channel may also be the site of action of the sulphonylureas, drugs used clinically to increase insulin secretion in type II diabetics, because in both mouse B-cells [3] and the B-cell line CRG-G1 [4] these drugs block K^+ fluxes through the ATP-sensitive K^+ channel.

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To date, all such studies have been carried out using B-cells derived from species other than man. We report here evidence for the presence of ATP-sensitive K^+ channels in the membranes of human pancreatic B-cells and for their inhibition by the sulphonylurea tolbutamide.

2. MATERIALS AND METHODS

Human pancreases were obtained from normoglycaemic, heart-beating cadaver organ donors (with permission) and islets isolated by collagenase digestion [5]. The secretory responses of human islets prepared in this way have been reported elsewhere [6]. After overnight tissue culture the islets were dispersed into single cells, plated onto glass coverslips and maintained in short-term tissue culture.

The following criteria indicate that our isolation methods yield viable human B-cells. (i) Isolated islets have low rates of basal insulin release and respond to glucose with a dose-dependent increase in insulin secretion and glucose oxidation [6]. (ii) Isolated single cells which were not in good condition were easily identifiable by the granulated ap-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

pearance of their cytoplasm, by their fragility (the patch membrane would spontaneously rupture to yield the whole-cell configuration) and by the occurrence of a large conductance (100 pS) K^+ channel at the resting potential in cell-attached patches. Such cells were not used in the present studies. The results we describe here were obtained from 6 successful preparations of human isolated B-cells (out of a total of 10).

Standard patch-clamp methods [7] were used to record single-channel currents from cell-attached or excised inside-out patches of plasma membrane. Full details of our recording system are given elsewhere [8]. The patch pipette was filled with (mM): 140 KCl, 5 $CaCl_2$, 5 $MgSO_4$, 5 NaHepes (pH 7.4). For cell-attached recordings the bath contained an extracellular solution (mM): 5 KCl, 135 NaCl, 5 $CaCl_2$, 5 $MgSO_4$, 5 NaHepes (pH 7.4). For inside-out patches the bath contained an intracellular solution (mM): 107 KCl, 2 or 5 $MgSO_4$, 1 $CaCl_2$, 11 EGTA + 11 Hepes (stock solution titrated to neutrality with KOH; total $[K^+]_i$ about 135 mM); pH 7.2, pCa = 8.0. Na_2ATP, KADP (Boehringer) or tolbutamide (Sigma) were added as indicated. A 1 M stock solution of K-tolbutamide was made by dissolving the drug in 1 M KOH. Intracellular solutions were exchanged using a rapid flow method [9]. All experiments were carried out at room temperature (20–23°C).

3. RESULTS

Currents flowing through single ATP-sensitive K^+ channels recorded at −60 mV from an inside-out membrane patch excised from a human B-cell are illustrated in fig.1A. In this configuration the cytoplasmic membrane surface is exposed to the bath solution. As shown in fig.1A, the addition of 1 mM ATP to the intracellular (bath) solution substantially inhibited channel activity, indicating that the channel is ATP-sensitive. Inhibition was reversed when ATP was removed (not shown). This result was observed in each of 4 patches (2 preparations).

The relationship between the potential across the patch membrane and the amplitude of the single-channel current is given in fig.1B. Single-channel currents reversed at approx. 0 mV, as expected for a K^+-selective channel exposed to symmetrical K^+ concentrations. Under these conditions, the single-

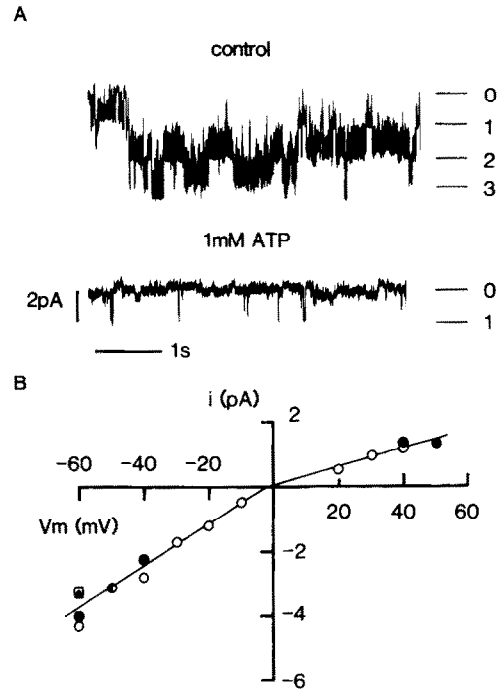

Fig.1. (A) ATP inhibition of single K^+ channel currents. Single-channel currents recorded at a membrane potential of −60 mV from an inside-out patch of human B-cell membrane exposed to intracellular solution before (above) and after (below) the addition of 1 mM ATP. The number of channels open is indicated to the right of each trace. (B) Mean current-voltage relation of the ATP-sensitive K^+ channel. Abscissa: membrane potential (mV); ordinate: single-channel current amplitude (pA). The symbols refer to different cells (n = 4); (●) cell illustrated in A. The lines are fitted using a least-squares method and have slopes of 64 pS (at negative potentials; correlation coefficient, 0.96) and 29 pS (at positive potentials). The currents reverse at an extrapolated potential of −2 mV.

channel conductance measured for inward currents was 64 pS, close to that found for ATP-sensitive channels in other cells [1,2,10,11].

The kinetics of the ATP-sensitive K^+ channel qualitatively resemble those reported for rodent B-cells [1,2] in consisting of bursts of channel openings separated by long interburst intervals (figs 1–4). At least two closed states are therefore indicated. Fig.1 further suggests that ATP reduces channel activity by decreasing the number of openings per burst. No effect on the amplitude of the single channel currents was observed.

The sulphonylureas constitute a selective blocker of ATP-sensitive K^+ channels [3]. Therefore, we tested the ability of these drugs to block ATP-sensitive K^+ currents in human B-cells (2 patches from different preparations). Tolbutamide (1 mM) applied to the intracellular membrane surface rapidly produced a complete block of single-channel currents (fig.2). Channel activity only partially recovered on removal of the drug; this does not imply that the effect of tolbutamide is only partly reversible, however, as we found channel activity runs down with time in isolated patches from human B-cells (as is found for other ATP-sensitive K^+ channels [12,13]). In an attempt to avoid the problem of rundown, we also tested tolbutamide on an inside-out patch in the presence of 0.1 mM ATP + 0.1 mM ADP (fig.3). Stable channel activity was found in this control solution; others have also reported that ATP is required to maintain channel activity in B-cells [3,11]. In the presence of these concentrations of ATP and ADP, 1 μM tolbutamide was ineffective, 10 μM almost, and 100 μM totally, inhibited channel activity. These results were fully reversible and could be repeated several times in the same patch. These preliminary observations are consistent with the dose-response curve reported for mouse B-cells where half-maximal inhibition is produced by 7 μM tolbutamide [3].

ATP-sensitive K^+ channel currents were also recorded from cell-attached patches on intact cells exposed to glucose-free solution (fig.4A). The mean conductance, measured over the linear region of the current-voltage relation between pipette potentials of 80 to -20 mV, was 56 pS (4

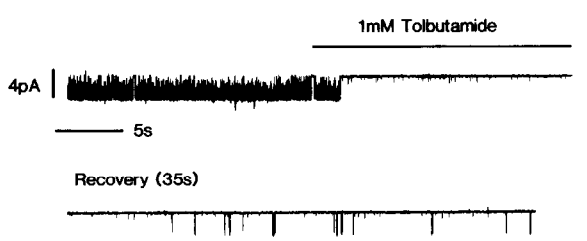

Fig.2. Effect of tolbutamide on single ATP-sensitive K^+ channel currents. Single-channel currents were recorded at -60 mV from inside-out patches excised from human B-cells. Intracellular solution ± 1 mM tolbutamide as indicated. The lower trace was obtained 35 s after the upper.

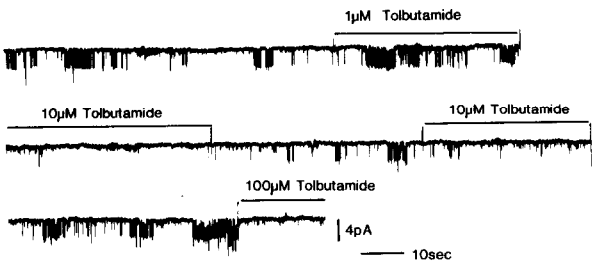

Fig.3. Effect of different tolbutamide concentrations on ATP-sensitive K^+ channel currents. Single-channel currents recorded at -60 mV from an inside-out patch exposed to intracellular solution containing 0.1 mM Na_2ATP + 0.1 mM KADP. Tolbutamide was added as indicated by the bar. The traces are a continuous recording from the same patch. The upward deflections of the current trace are artefacts associated with the solution exchange [8].

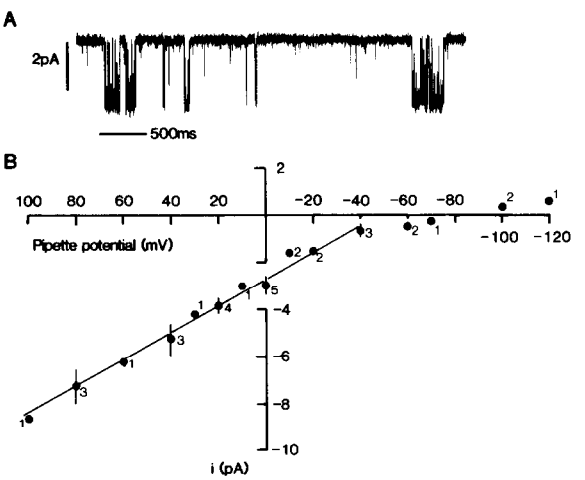

Fig.4. (A) Single-channel currents recorded at the resting potential (V_p = 0 mV) from a cell-attached patch on a human B-cell in standard external solution (no glucose). (B) Single-channel current-voltage relation measured from cell-attached patches on human B-cells. Abscissa: pipette potenial (mV), 0 mV = resting potential; ordinate: single-channel current (pA). Symbols indicate mean and vertical lines the SE. The number of samples at each potential is indicated adjacent to the symbol.

preparations) similar to that found for the isolated patch. Reversal of single-channel currents occurred at pipette potentials between -45 to -80 mV (mean -61 ± 9 mV; n = 4, 4 preparations; E_{rev} was extrapolated in 2 patches). This value is somewhat less than that found for rodent

B-cells (approx. −70 mV [1,2]) suggesting that either $[K^+]_i$ is higher in human B-cells or that the resting potential is on average around 10 mV more positive (i.e. about −60 mV). In healthy cells, ATP-sensitive K^+ channel currents constituted most of the channel activity observed at the resting potential but the amount of this activity was very variable. We also observed a large conductance K^+ channel (100 pS) activated at positive membrane potentials, similar to that reported in rodent B-cells [1].

4. DISCUSSION

The main finding we report here is the presence of an ATP-sensitive K^+ channel in the membranes of human B-cells. This channel was identified from its inhibition by millimolar ATP, its conductance, permeability to K^+, characteristic burst kinetics and block by tolbutamide. The dominant activity of the ATP-sensitive K^+ channel in intact cells exposed to glucose-free solutions suggests that, as in rodents [1,2], this channel may make a major contribution to the resting membrane K^+ permeability. If our preliminary observation that the ATP-sensitive K^+ channel in human B-cells is inhibited by tolbutamide is confirmed by further experiments, this might account for the hypoglycaemic action of the sulphonylureas in man.

Although the results presented here were obtained from a relatively few number of patches, we felt it important to report them because of the lack of any electrophysiological data on human B-cells. Our findings provide additional support for the view that models of insulin secretion derived from rodent islets may be applicable to man.

It is well established that glucose-stimulated insulin release is impaired in type II diabetes. Since sulphonylureas increase insulin secretion in type II diabetes and inhibit the ATP-sensitive K^+ channel, it may be that the primary lesion in at least some type II diabetics is a defect in the regulation of the ATP-sensitive K^+ channel. Thus it seems possible that in these diabetics the ATP-sensitive K^+ channel may be closed by sulphonylureas but not, as in normal subjects, by glucose metabolism. In this context it is interesting that futile cycles of ATP consumption have been reported for hepatocytes isolated from early type II diabetics [14]. We suggest that, in at least some patients, type II diabetes may result from either (i) a failure to generate the second messenger (possibly ATP) that links metabolic events to channel inhibition or (ii) a defect in the channel (or associated control proteins) which results in an inability to recognise the second messenger. Studies using B-cells isolated from type II diabetics will be required to test these hypotheses.

ACKNOWLEDGEMENTS

We thank Drs D. Gray and D.E. Harrison for preparing isolated human B-cells in preliminary experiments and Drs S.J.H. Ashcroft and R.C. Turner for their comments on the manuscript. This work was supported by the British Diabetic Association, the Wellcome Trust, the Medical Research Council, Nordisk UK and the Royal Society. F.M.A. is a Royal Society 1983 University Research Fellow.

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